Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A mobile phone-based approach to detection of hemolysis

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ARTICLE INFO

Article history:
Received 5 June 2016
Received in revised form
6 August 2016
Accepted 11 August 2016

Keywords:
mHealth
Hemolysis
Preeclampsia
HELLP syndrome
Blood analysis

ABSTRACT

Preeclampsia and HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome are pregnancy-related complications with high rates of morbidity and mortality. HELLP syndrome, in particular, can be difficult to diagnose. Recent work suggests that elevated levels of free cell hemoglobin in blood plasma can, as early as the first trimester, potentially serve as a diagnostic biomarker for impending complications. We therefore developed a point-of-care mobile phone–based platform that can quickly characterize a patient's level of hemolysis by measuring the color of blood plasma. The custom hardware and software are designed to be easy to use. A sample of the whole blood ($\sim$10 μL or less) is first collected into a clear capillary tube or microtube, which is then inserted into a low-cost 3D-printed sample holder attached to the phone. A 5–10 min period of quiescence allows for gravitational sedimentation of the red blood cells, leaving a layer of yellowish plasma at the top of the tube. The phone camera then photographs the capillary tube and analyzes the color components of the cell-free plasma layer. The software converts these color values to a concentration of free hemoglobin, based on a built-in calibration curve, and reports the patient's hemolysis level: non-hemolyzed, slightly hemolyzed, mildly hemolyzed, frankly hemolyzed, or grossly hemolyzed.. The accuracy of the method is $\sim$1 mg dL^{-1}. This phone-based point-of-care system provides the potentially life-saving advantage of a turnaround time of about 10 min (versus 4+hours for conventional laboratory analytical methods) and a cost of approximately one dollar USD (assuming you have the phone and the software are already available).

1. Introduction

Pregnancy-related complications are the fourth-leading cause of death in developing countries (World Health Organization, 2005), where expecting mothers are 36 times more likely to be affected by complications than are mothers in developed countries (World Health Organization, 2015). Major causes of maternal and neonatal deaths include preeclampsia, a hypertensive pregnancy disorder, and HELLP syndrome (Hemolysis, Elevated Liver enzymes, and Low Platelet count), an associated complication. Hemolysis is the rupturing of red blood cells, which release hemoglobin into the blood plasma. According to the Preeclampsia Foundation (2013), the mortality rate of HELLP syndrome has been reported to be as high as 25%. Overall perinatal mortality from HELLP syndrome (stillbirth plus neonatal death) ranges from 8 to 60% (Turgut et al., 2010).

HELLP syndrome is often difficult to diagnose because its symptoms (headache, nausea, blurry vision) are non-specific and can be mistaken for gastritis, flu, acute hepatitis, gall bladder disease, or other conditions. According to the American Congress of Obstetricians and Gynecologists (ACOG), preeclampsia is diagnosed mainly by new-onset hypertension (blood pressure higher than 140/90) in the second half of pregnancy in combination with new-onset proteinuria (excretion of more than 300 mg protein over a 24-h period) (American College of Obstetricians and Gynecologists, 2013). HELLP syndrome can progress very quickly, potentially resulting in multiple-organ failure, coma, or death in as little as three hours. Thus, rapid and reliable disease diagnosis is critical for patient survival.

Recent findings show that blood plasma samples from preeclamptic women contain elevated levels of cell-free plasma hemoglobin (Hgb) that could serve as a diagnostic biomarker from even the first trimester. The critical sequence of events begins with fetal hemoglobin leaking over to the maternal circulation system. The resulting protein increase causes oxidative damage to the placental barrier (Hansson et al., 2015) and reduces the availability of free NO, which in turn results in vasoconstriction. Examination of the placenta and maternal endothelium indicates that oxidative stress induced by the fetal hemoglobin contributes majorly to the pathology of preeclampsia (Hansson et al., 2015). In preeclampsia, HELLP, and eclampsia, and the coagulation system are both activated by the damaged endothelium, which may in turn cause subacute/acute disseminated intravascular coagulation (Thachil and Toh, 2009). In this condition, a dysregulation of coagulation

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http://dx.doi.org/10.1016/j.bios.2016.08.030
0956-5663/

and fibrinolysis results in lower platelet counts and fibrinogen levels and greater consumption of antithrombin. Fibrin gradually forms, especially in the kidneys and the liver (Hansson et al., 2015). In this way, acutely high levels of free Hgb contribute to kidney damage (Hansson et al., 2015).

With the abovementioned series of events, elevated levels of free-cell hemoglobin can be measured in the first trimester. Pre-eclamptic women have 53% higher cell-free plasma hemoglobin concentrations than healthy pregnant women (5.51 ± 0.56 mg dL^{-1} versus 3.62 ± 0.37 mg dL^{-1}) (Sandrim et al., 2010). Due to these higher Hgb concentrations, preeclamptic women also consume 63% more nitric oxide (NO). Later in pregnancy, the high Hgb levels correlate with high blood pressure in preeclamptic women (Hansson et al., 2015). The same correlation can be made in other hemolytic diseases such as autoimmune hemolytic anemia, sickle cell anemia, and malaria, which are also known to cause kidney disease (Bunn et al., 1977). High concentrations of hemoglobin in plasma can result in multiple-organ failures and potentially death. For all of these conditions, then, a cost-effective, compact, point-of-care analyzer for detecting Hgb in blood plasma would be of great benefit (Archibong et al., 2015; Adiga and Yogish, 2016; Simundic et al., 2010).

Conventional methods for detecting hemolysis rely on spectrophotometric determinations or sometimes visual estimations of a clinical quantity known as the hemolysis index (HI). A patient's blood sample is typically sent to automated laboratory, where the sample is centrifuged and the separated blood plasma is analyzed. The laboratory instruments are bulky (Dolci and Panteghini, 2014; Thomas, 2013) and turnaround times may be long (on the order of hours) relative to the urgency of some medical conditions. Delays in processing also increase the likelihood of cell lysis occurring in the sample during the time between collection and analysis, thus potentially confounding measurements of Hgb concentrations and other medically significant quantities. Recent advances in microfluidics (e.g., Crowley and Pizziconi, 2005) have been applied to the isolation of blood plasma but large, expensive spectrophotometric instruments are still required to measure analyte concentrations. Clinically, an additional diagnostic challenge is that hemolysis-related events are often inferred from the concentrations of other blood constituents rather than being assessed from direct measurements of Hgb itself.

For some types of chemical analyses, mobile phones provide an ideal analytical platform for a fully automated, point-of-care tool (Breslauer et al., 2009; Zhu et al., 2011). For example, the Ozcan Laboratory has developed a mobile phone-based platform for microscopy and flow cytometry (Zhu and Ozcan, 2013; Zhu, 2011). Advances related to phone-based imaging include colorimetric assays and the development of a super-resolution sample imaging algorithm (Breslauer et al., 2009; Cui et al., 2008; Zheng et al., 2010)). Example medical applications include *E. coli* detection (Zhu et al., 2012) and urinalysis for detection of the prostate cancer marker PCADM-1 (GENTAG Inc and MacroArray Technologies, LLC, 2014). Examples of environmental applications include analyses of toxic trace elements and pesticides (Wei et al., 2014; Mei et al., 2016).

Mobile phones are also widely available worldwide and are easy to use for mobile health (mHealth) applications (Kahn et al., 2010). An additional benefit is the ability to transmit and receive information in real time. We therefore developed an mHealth platform for the prompt, quantitative, onsite assessment of in vivo hemolysis (i.e., free plasma hemoglobin) in a small, fresh sample of citrated blood. The method requires a high-quality mobile phone camera with a flashlight, a 3D-printed hardware accessory, and a custom image-processing app. The primary user-managed steps in the procedure are to (a) collect the blood sample, (b) wait for gravitational settling of the red blood cells, and (c) photograph the resulting plasma layer. The result is reported to the user as a category of hemolysis: non-hemolyzed, slightly hemolyzed, mildly hemolyzed, frankly hemolyzed, or grossly hemolyzed. The entire procedure requires about 10 min.

2. Design, materials, and procedures

2.1. Mobile lab: hardware and software design

The complete point-of-care system for hemolysis detection consists of a mobile phone fitted with an attached sample holder (Fig. 1). The prototype platforms used a Samsung Galaxy Nexus 19,250 phone and a Nokia Lumia 520 phone. Both use the Android mobile operating system. The Samsung has a five-megapixel camera with a built-in LED flashlight, and the Nokia phone is

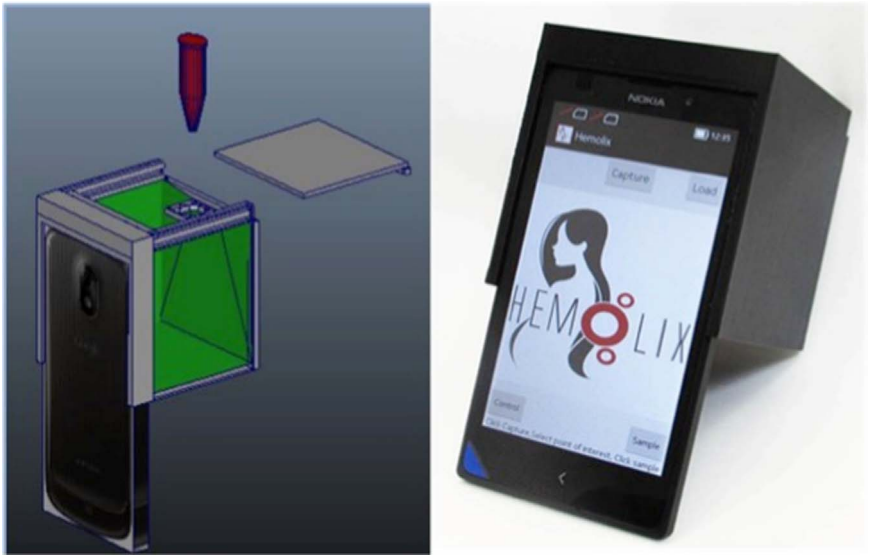

Fig. 1. Illustration and photograph of the mobile phone-based hemolysis-measurement platform. Left: The sample holder, with a ring slot and a lid, is connected to the back of the mobile phone. Right: One of the actual prototypes, with the 3D-printed sample holder attached to the phone. The screen on the phone shows the logo of the first software prototype ("Hemolix").

Please cite this article as: Archibong, E., et al., Biosensors and Bioelectronics (2016), http://dx.doi.org/10.1016/j.bios.2016.08.030